GLYOXAL AGAROSE

BACKGROUND OF THE INVENTION

The concept of immobilizing protein fragments and complexes including as illustrative antigens, antibodies, enzymes, etc., on gel structures has been described in the art. Gels are also widely used as support matrices for separating proteins by electrophoresis. However, no single gel developed heretofore could be used for both separating and immobilizing proteins. Ability to do either or both on one gel would provide options heretofore unavailable for design of new analytical methods. The gel substrates heretofore available and known have been too unstable, too reactive or of insufficient binding quality to be used for both separating and immobilizing proteins. Further, gels to be useful for electrophoretic separations in the studies within the scope of the field of use here of interest should be repeatably meltable, in the first instance to permit casting into slabs or sheets of a configuration useful for the specific electrophoretic separation contemplated, for example cross electrophoresis. Several known modifications of agarose commonly used for preparing affinity absorbents, were reproduced and their quality explored in an attempt to find one gel suitable for the contemplated electrophoretic procedures. All protein immobilizing gels tested were found to lose their activity and become resistant to melting due to cross-linking and consequent decomposition at melting temperatures. By exception, Parikh et al., *Methods in Enzymology* 34: 77–102 (1974) prepared for protein immobilization an aldehyde derivative of agarose by direct oxidation of agarose with periodate, which by our test was found to withstand melting. However, the binding capacity was objectionably low because of the small number of vicinal hydroxyl groups subject to partial oxidation.

The aldehyde rich glyoxal agarose of the present invention has excellent binding capacity and can be melted into sheets or slabs for multiple electrophoresis separations without complication. The aldehyde groups are essentially inert towards protein at neutral or acidic pH, but rapidly react with protein amino groups to form Schiff-base linkages. The inertness enables it to be used as a gel support for separating protein without binding at neutral pH, and high reactivity enables it to bind and immobilize the separated proteins simply through change to alkaline pH. The strong binding at alkaline pH can be reversed by neutralization, or it can be made irreversible by adding either by electrophoresing into the gel or immersing the cyanoborobydride gel in buffer with it. At pH near 10 the binding capacity is quite high—of the order of 2 to 3 mg of protein per mg of glyoxal agarose, as measured with both fibrinogen and cytochrome C, two proteins of widely differing molecular weight.

Thus it can be seen that the novel glyoxal agarose of this invention will have many and varied applications in immunochemical, enzymological and biochemical analysis and research to provide means for carrying out critical separations and analyses of complex biological specimens by solid phase methods. Mixtures of closely related substances may be separated on the gel, may be eluted or concentrated, or they may be permanently attached to the gel matrix to thereby facilitate identification of the substances and their possible relationships

to other identified substances in the mixture upon analysis.

A principal purpose of the invention is to provide a gel complex which is of such nature as to controllably bind specific proteins either reversibly, irreversibly, or not at all as may be selected by an investigator.

A first function is to selectively bind a desired protein to concentrate the selected protein from a dilute aqueous medium. This can be accomplished reversibly. That is, the protein may be recovered from the gel after its selective sorption, or it may be irreversibly bound to the gel, or it may so processed that a given protein is not bound at all to the novel gel of this invention. It is considered that the gel has the capacity to bind proteins through formation of Schiff base linkages—which base linkages are non-dissociable at alkaline pH of greater than about 9.5, but which bases are readily dissociated at neutral and acid pH. The inertness of the gel at neutral or acidic pH makes possible separation of protein from complex mixtures of them in positions and according to molecular weight range by either electrophoresis or by gel filtration. Once separated, the protein can be attached to the gel by making the solution alkaline, and the attachment made permanent by treating with cyanoborohydride. By controlled means it becomes feasible to sequentially sorb, desorb, mobilize and demobilize protein within a relatively narrow molecular weight range—repeated experiments providing reproducible profiles of their molecular weights and electrophoretic mobility, even when working with picomole size samples, 1×10^{-12}.

Once having separated the specific proteins of interest (illustratively) antigens and antibodies) detection, identification and assay of the isolate can be carried forward by known techniques.

The invention disclosed herein has been reduced to practice in detecting fibrinogen related antigens which have been heretofor non-specifically precipitated and most often denatured by the variable prior art means employed for their recovery. The sensitivity of the method of zonal immobilization of proteins permits detection and measurement of specific antigenic determinants in a 0.02 mg tissue specimen (microtome section 1 $cm^2\times2$ micron), where the specific antigenic determinant is that portion of the antigen molecule that determines the specificity of the antigen-antibody reaction.

Complex separations are potential through electrophoresis to produce a separated protein-gel fraction. This fraction may be immobilized (temporary) by a pH shift to a pH 10 with carbonates, or permanently by a second electrophoresis which provides intimate contact of the protein-gel fraction with a small amount of a reducing agent, preferably a cyanoborohydride ion. The Schiff base linkages are converted to stable alkyl amino linkages. The technique permits detection and assay of specific protein antigens thus separated.

The novel gel of this invention makes possible capture and separation by, if desired, repeated sequential sorption, desorbtion, mobilizing and immobilizing biologically germane protein bodies within very narrow ranges of molecular weight and to do so repetitively with reproducible results employing specimen samples of quantities in the picomole levels. The following examples are illustrative:

EXAMPLE 1

The product of this invention, herein referred to as glyoxal agarose (but which is also identified by the